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Ecto-5′-Nucleotidase (CD73) Deficiency in *Mycobacterium tuberculosis*-Infected Mice Enhances Neutrophil Recruitment

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The immune system needs safeguards that prevent collateral tissue damage mediated by the immune system while enabling an effective response against a pathogen. The purinergic pathway is one such mechanism and finely modulates inflammation by sensing nucleotides in the environment. Extracellular ATP is considered to be a danger signal leading to a proinflammatory response, whereas adenosine is immunosuppressive. CD73, also called ecto-5′-nucleotidase, occupies a strategic position in this pathway, as it is the main enzyme responsible for the generation of adenosine from ATP. Here, we explore the role of CD73 during tuberculosis, a disease characterized by an immune response that is harmful to the host and unable to eradicate *Mycobacterium tuberculosis*. Using CD73 knockout (KO) mice, we found that CD73 regulates the response to *M. tuberculosis* infection *in vitro* and *in vivo*. Mycobacterium-infected murine macrophages derived from CD73 KO mice secrete more keratinocyte chemoattractant (KC), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6) and release less vascular endothelial growth factor (VEGF) upon ATP stimulation than do those derived from wild-type (WT) mice. *In vivo*, CD73 limits the early influx of neutrophils to the lungs without affecting bacterial growth and dissemination. Collectively, our results support the view that CD73 fine-tunes antimycobacterial immune responses.

Tuberculosis (TB) is one of the most common infectious diseases worldwide, with 9 million new cases and 1.5 million deaths reported globally in 2014 (1). The success of *Mycobacterium tuberculosis*, the etiological agent of the disease, lies in its capacity to survive and replicate within phagocytes. This intracellular pathogen has developed a wide range of mechanisms to escape the bactericidal functions of macrophages and to modulate host immune responses (2). In particular, *M. tuberculosis* inhibits phagosome acidification (3), blocks autophagy (4), interferes with inflammasome activation (5), and inhibits apoptosis (6). Cell death is an important feature of tuberculosis. Upon phagocytosis by alveolar macrophages, the host response orchestrates the formation of well-organized cellular aggregates called granulomas. These structures contain the infection but paradoxically seem to promote it by inducing the infection of newly recruited nonactivated phagocytes (7). If the host fails to control *M. tuberculosis*, the center of the granuloma becomes necrotic and disintegrates, releasing the bacteria into the extracellular environment. By favoring necrosis instead of apoptosis, *M. tuberculosis* escapes from an ancestral immune defense mechanism and induces the infection of bystander phagocytes (6, 7), but necrosis also leads to the release of the intracellular content into the milieu.

The presence of intracytoplasmic molecules in the extracellular space has several consequences for the immune system. Many intracytoplasmic molecules are recognized as danger signals or damage-associated molecular patterns (DAMPs). For instance, it is well known that the purinergic pathway modulates immunity (8). ATP released either from dying cells or via pannexin and connexin hemichannels elicits many inflammatory responses, such as granule release by neutrophils, T-cell activation, cytokine and chemokine secretion by macrophages, the generation of reactive oxygen or nitrogen species, and dendritic cell maturation and migration (9). In the extracellular environment, ATP is rapidly metabolized to ADP and AMP by ecto-ATPases of the ectonucleoside triphosphate diphosphohydrolase family, which includes ectonucleoside triphosphate diphosphorylase 1 (CD39) (10). AMP is then converted to adenosine by ecto-5′-nucleotidase (CD73) (10). In contrast to extracellular ATP, adenosine is considered to be anti-inflammatory. For instance, the signaling of extracellular adenosine via the adenosine A_{2A} receptor inhibits the generation of free radicals and the secretion of inflammatory cytokines by neutrophils and macrophages (11–14) and represses the activation of Th1 and Th17 lymphocytes (15). In *M. tuberculosis*-infected human macrophages, activation of the adenosine A_{2A} receptor dampens the expression of inflammatory genes and promotes that of genes involved in tissue repair (13).

In the purinergic pathway, CD73 is pivotal in the conversion of proinflammatory ATP/ADP into immunosuppressive adenosine. CD73 is a glycosylphosphatidylinositol-linked surface protein expressed on subsets of T/B lymphocytes, myeloid cells, vascular endothelial cells, and some epithelial cells (16). The role of CD73 in cancer has been well described. CD73 is overexpressed in numerous types of cancer (17), and strategies that target CD73 may

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limit both tumorigenesis and metastasis (18–21). CD73 knockout (KO) mice also have strong antitumor immunity (22). However, the role of CD73 in infectious diseases has been poorly explored. CD73-deficient mice are more resistant to *Salmonella enterica* and *Helicobacter felis* infections and display a stronger inflammatory response than wild-type (WT) mice (23, 24). These observations are in contrast to those obtained upon infection with *Toxoplasma gondii*. After oral infection with *T. gondii*, CD73 KO mice are resistant to chronic toxoplasmosis in the central nervous system (25) but are highly susceptible to damage mediated by the immune system following intraperitoneal injection of the parasites (26). CD73 KO mice also showed a high rate of mortality in a model of sepsis (27). The role of CD73 in tuberculosis has never been explored. Here, we used both *in vitro* and *in vivo* models to investigate the effect of this pivotal component of the purinergic pathway on inflammatory responses during the course of *M. tuberculosis* infection in mice. Our findings may facilitate the design of new strategies to combat TB.

MATERIALS AND METHODS

Ethics statement. All animal experiments described in the present study were conducted at the Institut Pasteur according to European Union guidelines for the handling of laboratory animals (http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm) and were approved by the Institut Pasteur Animal Care and Use Committee and the Direction Sanitaire et Vétérinaire de Paris under permit number 00244. All experiments were subject to the three R's of animal welfare (refine, reduce, and replace).

Mice and infection. C57BL/6J mice were purchased from Janvier Labs, and CD73 KO mice inbred into the C57BL/6J background were a kind gift from Linda Thompson (Oklahoma Medical Research Foundation, Oklahoma City, OK, USA). Seven-week-old female CD73 KO or C57BL/6J mice were infected via the aerosol route. Aerosol challenge was performed with the H37Rv strain to obtain an inhaled dose of <150 CFU per lung. One day after infection, two mice were killed, and the numbers of CFU were determined.

Determination of CFU. Lungs and spleens were homogenized in phosphate-buffered saline (PBS) by using M tubes (Miltenyi Biotec). Serial dilutions were plated onto 7H11 agar plates supplemented with Panta (BD Biosciences). Colonies were counted after 21 days of incubation at 37°C.

Isolation and infection of bone marrow-derived macrophages (BMMs). Female 7- to 8-week-old C57BL/6J or CD73 KO mice were killed by cervical dislocation. Bone marrow cells were obtained by flushing of femurs. Cells were differentiated into macrophages with recombinant mouse macrophage colony-stimulating factor (M-CSF) (50 ng/ml; Life Technologies) for 5 days in RPMI containing 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin-streptomycin-neomycin (Gibco). Cells were then infected as described previously, at a multiplicity of infection of 1 (28). After 2 h of incubation, the cells were washed and incubated with fresh medium for 18 h in the presence or absence of 100 μM ATP (Sigma-Aldrich).

Histology. For histopathological analysis, aerosol-infected CD73 KO mice and WT mice were killed by cervical dislocation. Lungs were fixed in JB fixative (0.5% zinc acetate, 0.05% zinc chloride, and 0.05% calcium acetate in Tris buffer at pH 7) for 7 days and then embedded in low-melting-point paraffin (polyethylene glycol distearate; Sigma). To examine histological lesions, 4-μm-thick sections were cut and stained with hematoxylin-eosin (HE). For immunohistochemistry analyses, the following primary antibodies were used: anti-lymphocyte antigen 6 complex, locus G (anti-Ly6G) (clone A14748; Life Technologies) for neutrophils, anti-CD3 (clone A0452; Dako) for T lymphocytes, anti-CD45R (clone B220; Life Technologies) for B lymphocytes, and F4/80 (clone MF4800; Life Technologies) for macrophages.

Cell surface staining and flow cytometry. Lungs were perfused with 10 ml PBS. A single-cell suspension was obtained by using C tubes (Miltenyi Biotec), followed by incubation with 1 mg/ml of collagenase D (Roche) and 0.1 mg/ml of DNase (AplliChem) for 1 h at 37°C. Cells were washed and suspended in PBS containing 3% FBS. Erythrocytes were lysed in ammonium-chloride-potassium lysis buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM EDTA, H_2O), and cells were washed with PBS. Finally, cells were incubated with Fc-Block antibody (anti-CD16/CD32; BD Biosciences) for 15 min at 4°C and stained with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (MAbs) specific for CD8, CD11b, F4/80, and B220. Allophycocyanin (APC)-conjugated MAbs specific for CD3 and Ly6G; phycoerythrin (PE)-conjugated MAbs specific for CD4, CD11c, and CD73; and a PE-cyanine 7-conjugated MAb specific for CD45.2 were also used. All the MAbs were purchased from eBioscience. Cells were analyzed with a MACSQuant cytometer system (Miltenyi Biotec) and with FlowJo software (Treestar).

Bronchoalveolar lavage. After the mice were euthanized, lavage was performed by introducing 1 ml of sterile PBS into the lungs, followed by aspiration, reinjection, and reaspiration two additional times. The bronchoalveolar lavage (BAL) fluid was stored at −80°C prior to its use in enzyme-linked immunosorbent assays (ELISAs).

ELISA. Supernatants from uninfected cells and *M. tuberculosis*-infected cells were filtered through 0.22-μm-pore-size filters (Millipore Corp.). Concentrations of tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF) (R&D Systems), keratinocyte chemoattractant (KC), and interleukin-6 (IL-6) (Peprotech) were determined by an ELISA according to the manufacturer's instructions.

Statistical analysis. Data are expressed as means ± standard deviations. Statistical analyses were performed with Prism software (GraphPad Software Inc.), using the *t* test and one-way analysis of variance (ANOVA) with Tukey's *post hoc* test, as indicated in the figure legends. A P value of <0.05 was considered to be significant.

RESULTS

CD73 modulates the response of *M. tuberculosis*-infected macrophages upon ATP stimulation. In human macrophages infected with *M. tuberculosis*, extracellular ATP is rapidly converted into AMP and adenosine, leading to an impairment of the secretion of inflammatory mediators, such as TNF-α, and stimulating the release of molecules involved in wound healing, such as VEGF (13). We first evaluated whether these responses are conserved in murine macrophages. Bone marrow-derived macrophages (BMMs) were infected with virulent *M. tuberculosis* strain H37Rv and stimulated with 100 μM ATP, and cytokine levels were assessed 18 h later. Such concentrations of ATP are observed under pathological conditions such as inflammation (9, 23) and may be reached in cases of tuberculosis. Consistent with results for human macrophages, in the presence of ATP, the secretion of TNF-α by *M. tuberculosis*-infected BMMs decreased, whereas that of VEGF increased (Fig. 1A).

In murine peritoneal macrophages and in human monocytes, extracellular ATP negatively regulates Toll-like receptor (TLR) signaling and suppresses the secretion of IL-12p40 and TNF-α, in part through the generation of adenosine (29, 30). Given that BMMs express CD73 (Fig. 1B), we evaluated whether CD73 modulates the secretion of cytokines involved in tuberculosis immunity, namely, TNF-α, IL-6, and VEGF (31–33). Upon incubation with ATP, *M. tuberculosis*-infected BMMs derived from CD73 KO mice secreted more TNF-α and IL-6 and less VEGF than did BMMs derived from WT mice (Fig. 1C). These results suggest that CD73 regulates the inflammatory response of infected BMMs stimulated with ATP, most likely through the formation of adenosine.

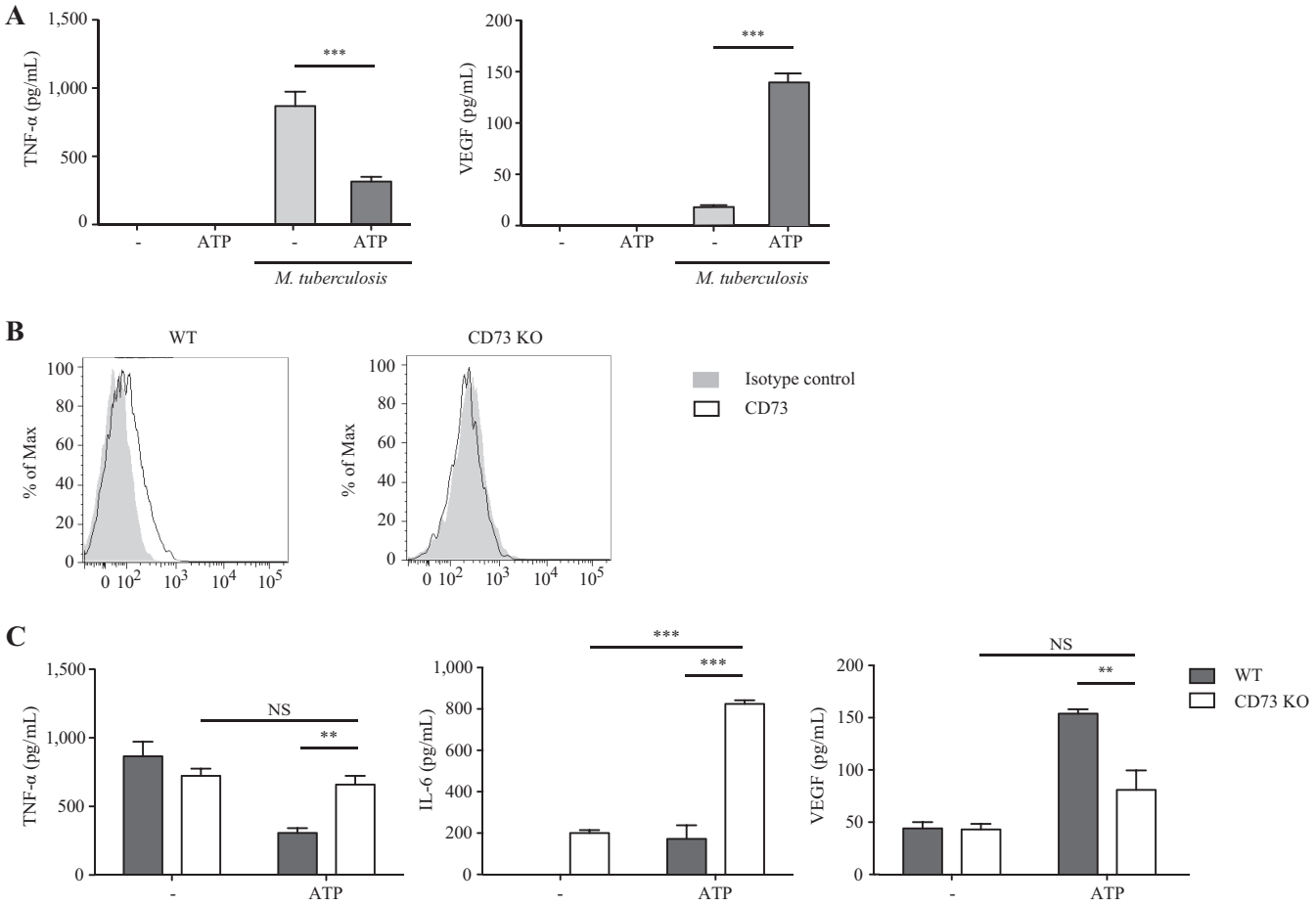

FIG 1 CD73 regulates the response of *M. tuberculosis*-infected BMMs to ATP stimulation. (A) Secretion of VEGF and TNF-α 18 h after *M. tuberculosis* infection in the culture supernatants of BMMs that were either unstimulated (light gray) or stimulated with 100 μM ATP (dark gray) was quantified by an ELISA. (B) Flow cytometric analyses of CD73 expression in infected BMMs isolated from WT (left) or CD73 KO (right) mice. The isotype control and CD73 staining are shown. (C) TNF-α, IL-6, and VEGF secretion by *M. tuberculosis*-infected BMMs incubated in the presence or absence of 100 μM ATP was measured by an ELISA. Error bars indicate standard errors of the means. One-way analysis of variance was performed with Tukey's multiple-comparison *post hoc* analysis. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant. Data are representative of results from three independent experiments.

CD73 is not required for macrophages to kill mycobacteria. TNF-α and gamma interferon (IFN-γ) stimulate the bactericidal functions of murine macrophages by generating reactive nitrogen intermediates (34–36). As TNF-α secretion was modulated by CD73 expression, we investigated the capacity of BMMs from CD73 KO mice to secrete nitric oxide (NO). BMMs from CD73 KO or WT mice were prestimulated with IFN-γ and infected in the presence or absence of ATP. The NO concentration in the supernatant was quantified 18 h later. As shown in Fig. 2A, the level of NO did not depend on CD73 expression. As a control, CD73 did not influence the uptake of *M. tuberculosis* by untreated or IFN-γ-activated macrophages after 2 h of infection (see Fig. S1A in the supplemental material). In line with these results, CFU numbers were similar for CD73 KO and WT BMMs during infection, even in the presence of 100 μM ATP (Fig. 2B). High concentrations of ATP (3 to 5 mM), well above physiological concentrations, have been reported to limit bacterial growth in macrophages through the induction of phagosome-lysosome fusion and autophagy (37–41). We therefore investigated whether CD73 could regulate bacterial killing in the presence of 3 mM ATP; we found no evidence of a role for this molecule (see Fig. S1B in the supplemental material). Thus, CD73 did not improve the capacity of unstimulated or IFN-γ-activated BMMs to control the growth of intracellular *M. tuberculosis*.

CD73 KO mice are as susceptible to *M. tuberculosis* infection as wild-type mice. We next investigated whether CD73 was implicated in the control of *M. tuberculosis* infection *in vivo*. WT mice and CD73 KO mice were infected with a low dose of bacteria by the aerosol route and then monitored for survival, weight loss, and bacterial burden. Over 84 days of infection, CD73 KO mice and WT mice gained weight in a similar manner, and all mice were still alive at the end of the experiments (data not shown). As shown in Fig. 3A, no significant differences between the bacterial burdens in the lungs of CD73 KO and WT mice at 21, 42, and 84 days postinfection were observed. The numbers of CFU obtained from the lungs were similar for the two groups during the acute phase of infection (days 1 and 7) (see Fig. S2 in the supplemental material). CD73 also seemed to play no role in mycobacterial dissemination, as the numbers of CFU obtained from the spleens of CD73 KO mice and WT mice were similar (Fig. 3B).

The lungs of *M. tuberculosis*-infected CD73 KO mice are transiently more inflamed and contain more neutrophils than the lungs of their WT counterparts. Tuberculosis is characterized

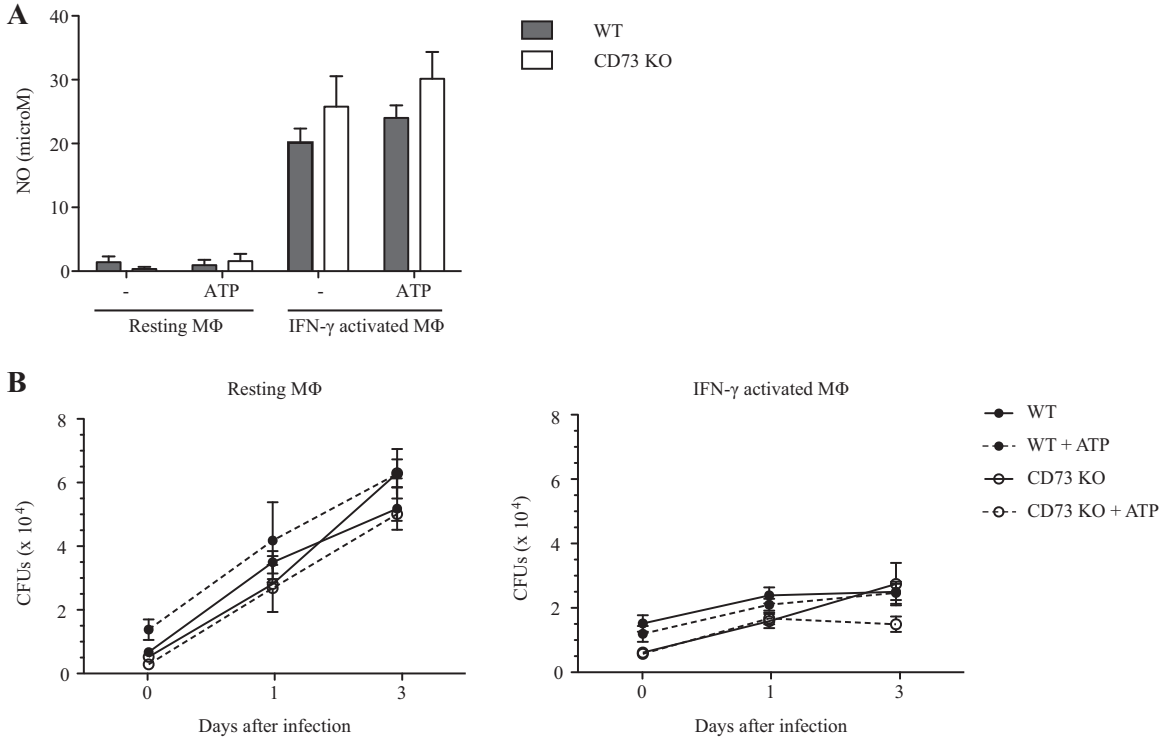

FIG 2 CD73 is not required for murine macrophages (MΦ) to kill mycobacteria. BMMs from *M. tuberculosis*-infected WT or CD73 KO mice were activated with IFN-γ (or were left untreated) and then incubated in the presence or absence of ATP. (A) NO secretion was quantified 18 h after infection. (B) Infected BMMs from WT or CD73 KO mice were incubated in the presence or absence of ATP. The numbers of CFU were determined at 0, 1, and 3 days postinfection. Error bars indicate standard errors of the means. Data are representative of results from three independent experiments.

by extensive inflammation (32). *M. tuberculosis*-infected macrophages secrete inflammatory cytokines and orchestrate the recruitment of immune cells, leading to the formation of granulomas (32). As CD73 dampens the inflammatory response of infected BMMs, we hypothesized that CD73 limits the pathology of tuberculosis *in vivo*. Lung sections of CD73 KO and WT mice were analyzed 21, 42, and 84 days after inoculation. We observed large differences between the two strains at 21 days postinfection (Fig. 4), especially regarding the severity and organization of inflammatory lesions and the density of neutrophil infiltration. Comparatively, WT mice displayed globally less severe inflammatory lesions, rare neutrophil infiltrates randomly distributed in the lung parenchyma, and lymphocytes surrounding bronchi/bronchioles and large blood vessels. In contrast, lesions were more severe in CD73 KO mice and were characterized by extensive neutrophil infiltration with no particular organization at 21 days postinfection. We observed no difference in T/B-lymphocyte and macrophage infiltration between the two strains (see Fig. S3 in the supplemental material). In contrast, at days 42 and 84 of infection, we detected no differences between WT and CD73 KO mice (see Fig. S4 in the supplemental material). Indeed, inflammatory lesions of the same nature and severity were present in both strains,

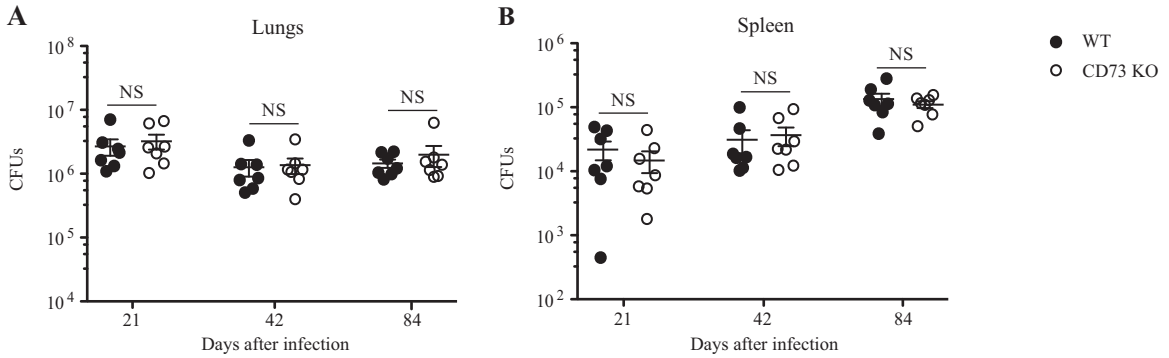

FIG 3 Growth and dissemination of *M. tuberculosis* are not affected by CD73 deficiency. Mice were infected via the aerosol route with <150 CFU of *M. tuberculosis* H37Rv. Bacterial loads in the lungs (A) and spleen (B) of WT and CD73 KO mice were measured at different time points postinfection. Seven mice per group were included. Error bars indicate standard errors of the means. Data are representative of results from three independent experiments. One-way analysis of variance was performed with Tukey's *post hoc* test. NS, not significant.

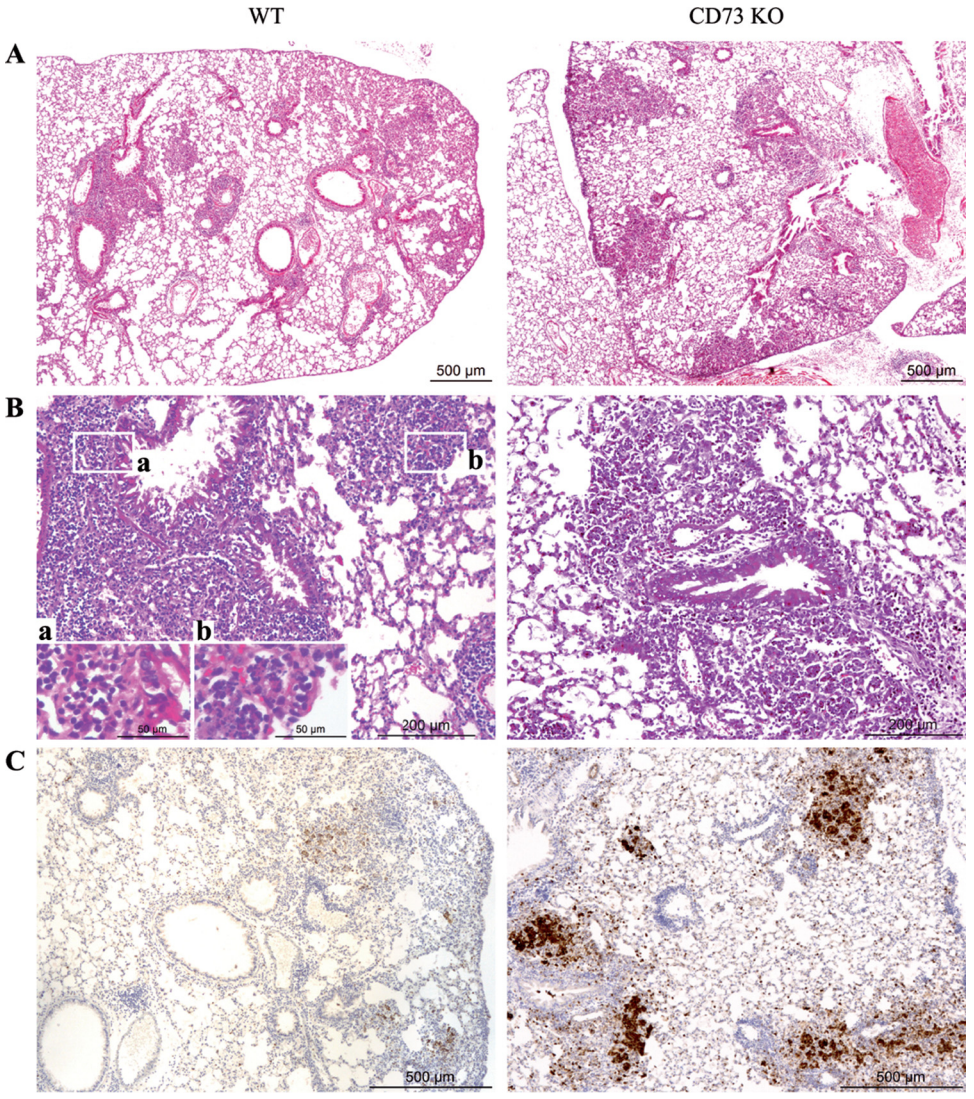

FIG 4 CD73 regulates pulmonary infiltration by neutrophils during *M. tuberculosis* infection. (A) At 21 days postinfection, WT and CD73 KO mice display differences in the severity and organization of inflammatory lesions. (B) At a higher magnification, lesions in WT mice have a specific organization: lymphocytes are at the periphery of bronchioles and blood vessels (a), whereas neutrophils and macrophages are at a distance (b). In contrast, lesions in CD73 KO mice are not organized in any particular way. (C) Immunohistochemistry analysis reveals small infiltrates of Ly6G-positive neutrophils in WT mice and large infiltrates in CD73 KO mice. Data are representative of results from three independent experiments.

characterized by the presence of multifocal-to-coalescing infiltrates of foamy macrophages associated with lymphocytes and rarely with neutrophils. Lesions were more severe at day 84 than at any other time point in both groups of mice.

We analyzed lung immune cells by flow cytometry to investigate this exacerbated inflammatory context further. Consistent with our histopathological observations, at day 21 postinfection, the percentage of polymorphonuclear neutrophils in the lungs was significantly higher in CD73 KO mice than in WT mice (Fig. 5). The percentages of $CD4^+$ and $CD8^+$ T lymphocytes, B cells, and dendritic cells in the lungs of WT and CD73 KO mice infected with *M. tuberculosis* were similar (Fig. 5A). In *M. tuberculosis*-infected mice, the percentage of alveolar macrophages in the lungs tended to be lower in the CD73 KO strain than in the WT strain (Fig. 5A); however, we observed a similar trend in noninfected mice (Fig. 5B).

Increased TNF-α, IL-6, and KC levels in CD73 KO mice after *M. tuberculosis* infection. We then evaluated whether the greater influx of neutrophils in CD73 KO mice was accompanied by a differential expression of the cytokines studied *in vitro* and of KC, a potent chemoattractant of neutrophils (42). We analyzed the expression of IL-6, KC, TNF-α, and VEGF in the bronchoalveolar lavage fluid of WT and CD73 KO mice after 21 days of infection. The concentrations of KC, IL-6, and TNF-α were higher in the lungs of CD73 KO mice than in those of WT mice, whereas VEGF levels were similar in the two groups. (Fig. 6A). KC expression may thus account for the recruitment of larger numbers of neutrophils to the lungs of CD73 KO mice after 21 days postinfection.

We next investigated whether *M. tuberculosis*-infected BMMs could be a source of KC and whether the secretion of this molecule was influenced by CD73. BMMs derived from CD73 KO mice

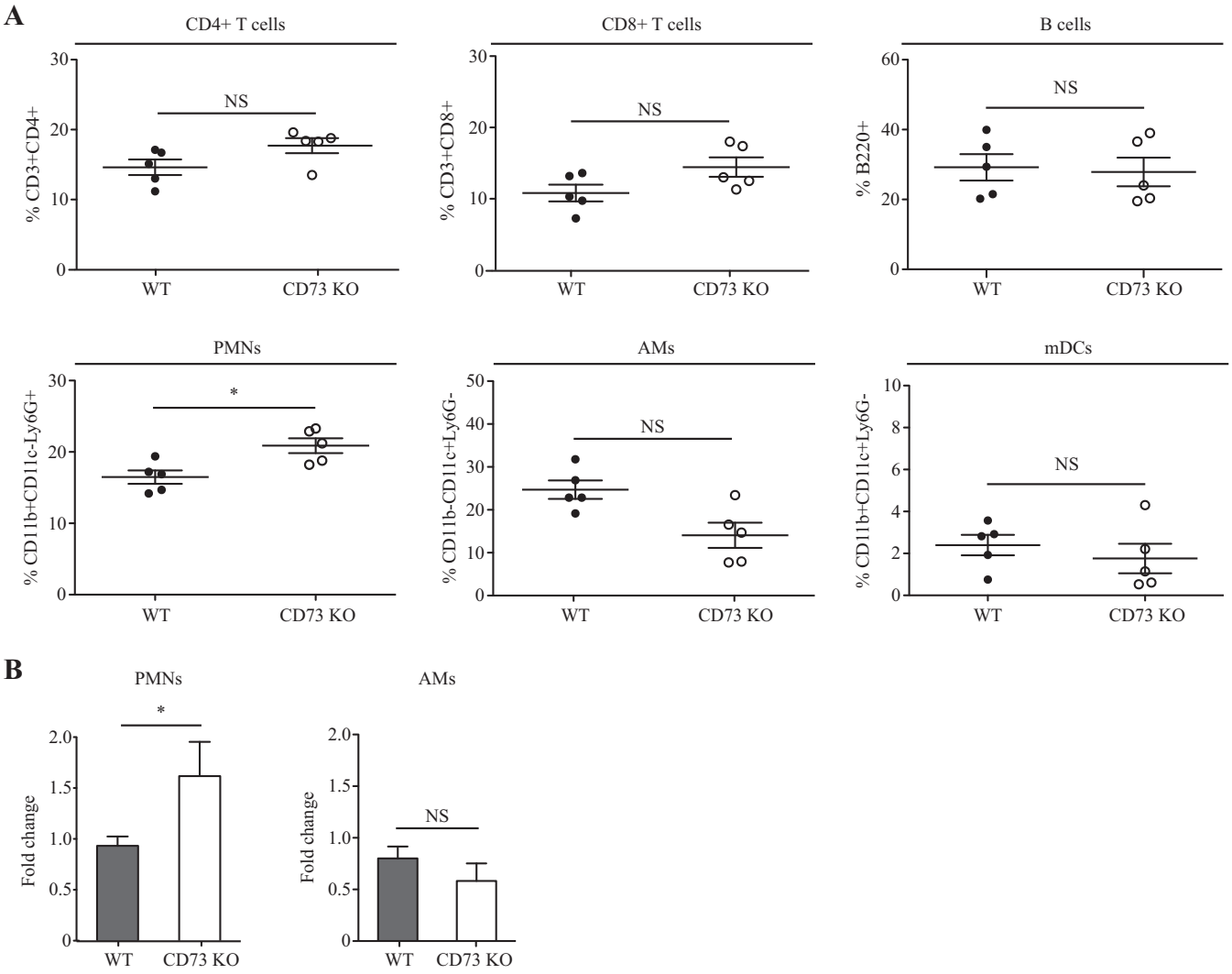

FIG 5 Following *M. tuberculosis* infection, higher levels of neutrophil influx into the lungs were observed in CD73 KO mice than in WT mice. (A) Different cell populations in the lungs of WT and CD73 KO mice 21 days after infection were quantified by flow cytometry. (B) Fold change of the percentages of polymorphonuclear neutrophils (PMNs) and alveolar macrophages (AMs) between uninfected mice and mice infected 21 days later. mDCs, myeloid dendritic cells. Five mice per group were included. Error bars indicate standard errors of the means. Data are representative of results from two independent experiments. *, $P < 0.05$; NS, not significantly different from the results for WT mice according to an unpaired *t* test with a *P* value cutoff of <0.05.

secreted more KC upon mycobacterial infection than did BMMs derived from WT mice (Fig. 6B).

DISCUSSION

Immune responses must be finely regulated to avoid excessive tissue damage. Purinergic signaling is one such regulatory pathway. Extracellular ATP is considered to be proinflammatory, whereas adenosine dampens inflammation (10). In the current study, we evaluated the role of CD73, the rate-limiting enzyme that converts AMP to adenosine. We show for the first time that CD73 modulates the response of *M. tuberculosis*-infected BMMs upon stimulation with ATP *in vitro* and *in vivo*, in *M. tuberculosis*-infected mice. *M. tuberculosis*-infected BMMs derived from CD73 KO mice secreted less VEGF and more KC, TNF-α, and IL-6 than did those derived from WT mice. *In vivo*, the levels of these cytokines (with the exception of VEGF) were also higher in CD73 KO mice than in WT mice after 21 days postinfection. However, CD73 deficiency does not affect mycobacterial growth *in vitro* or *in vivo*. In contrast, CD73 impairs bacterial clearance in the livers of mice infected with *S. enterica* serovar Typhimurium and in the spleens of those infected with *H. felis* (23, 24). In fact, CD73 rather seems to play a transitory role in the early homing of innate immune cells, and notably neutrophils, to the lung parenchyma and to tissues damaged during *M. tuberculosis* infection. Our results show that CD73 downregulates KC secretion in BMMs and *in vivo*, probably explaining the increased neutrophil recruitment in CD73 KO mice upon *M. tuberculosis* infection.

The role of neutrophils during tuberculosis remains controversial and seems to be a double-edged sword. Neutrophils are readily infected by the bacillus (43), but their capacity to kill *M. tuberculosis* directly has not been convincingly demonstrated (44). However, phagocytosis of apoptotic neutrophils by macrophages decreases the viability of intracellular bacteria (45), and the uptake

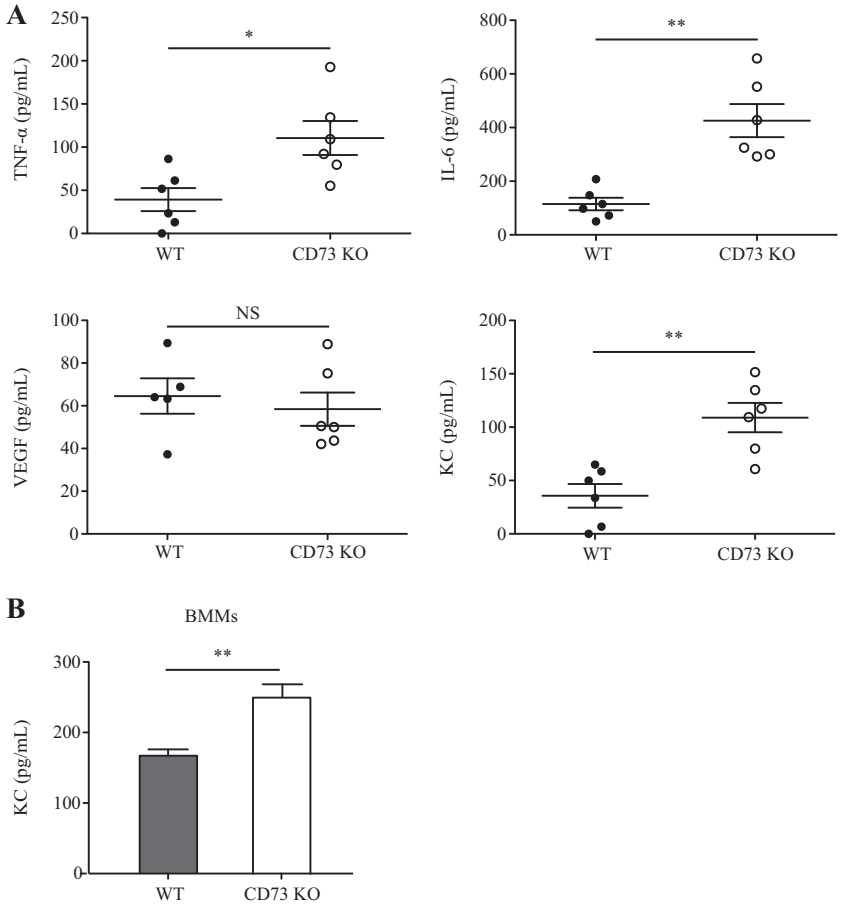

FIG 6 KC, TNF-α, and IL-6 levels increase upon infection *in vivo* in CD73 KO mice. (A) Cytokine levels in bronchoalveolar lavage fluid from WT and CD73 KO mice 21 days after infection by the aerosol route were determined. Five or six mice per group were studied. (B) KC levels in the supernatants of CD73 KO and WT BMMs 18 h after *M. tuberculosis* infection were quantified by an ELISA. Error bars indicate standard errors of the means. **, $P < 0.01$; *, $P < 0.05$; NS, not significantly different from the results for WT mice according to an unpaired *t* test with a *P* value cutoff of <0.05. The data shown are representative of results from two independent experiments.

of apoptotic neutrophils by dendritic cells facilitates the activation of $CD4^+$ T lymphocytes (46). During *M. tuberculosis* infection *in vivo*, neutrophils are mainly involved in immune-mediated lung damage (32). Neutrophils are recruited at high numbers in susceptible mouse strains, and their depletion has a protective effect and limits the bacterial load (47, 48).

The recruitment of neutrophils to the site of inflammation is influenced by the purinergic pathway, in particular by CD39 and CD73. Neutrophil trafficking during lipopolysaccharide (LPS)- or bleomycin-induced lung injury is highly active in mice deficient for CD39 or CD73 or in mice treated with specific inhibitors of these two enzymes (49). Neutrophil influx has also been observed in CD73 KO mice infected with *T. gondii* (26). Although CD73 is widely expressed by immune cells, including macrophages, its expression at the surface of endothelial cells may be important for the control of neutrophil influx and tissue damage upon *M. tuberculosis* infection. During hypoxia, adenosine is formed at the surface of endothelial cells by CD39 and CD73, stimulates the functions of the endothelial barrier (50), and triggers an anti-inflammatory response (51, 52). Indeed, the activation of neutrophils via the adenosine A_{2A} and A_{2B} receptors acts as an antiadhesive signal for the microvascular endothelium (51).

In our experiments, the strong recruitment of neutrophils in CD73 KO mice was transitory and observed only at 21 days postinfection. One possible explanation for this finding is that adenosine is rapidly converted to inosine by adenosine deaminase (ADA) during the chronic phase of the disease, thus limiting the effect of CD73. The concentration of ADA is high in the serum of patients with pleural tuberculosis (53), and ADA may be a useful biomarker of tuberculosis. It remains possible that alkaline phosphatase (AP), which hydrolyzes AMP to adenosine, plays a compensatory role in CD73 KO mice (54). In human lungs, CD73 and nonspecific AP mRNAs are detected mainly in the higher and lower airways, respectively (54). Interestingly, the activity of AP is increased in tuberculosis patients (54, 55).

Our observations in mice raise questions about the potential role of CD73 in humans. Unlike granulomas in humans with tuberculosis, mouse granulomas are not usually necrotic or hypoxic. Necrosis is a large source of extracellular nucleotides, and hypoxia regulates the expression of CD73 (56). Further studies, such as those in nonhuman primates, are thus needed to shed light on the role of CD73 during tuberculosis.

ACKNOWLEDGMENTS

We declare that we have no conflicts of interest.

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